UNRAVELLING PHOTOSYNTHESIS AND NITROGEN ASSIMILATION IN *CLADOCOPIUM* ALGAL INHABITING MARINE FRAGINAE (CARDIIDAE) COCKLES

By

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Abstract

Photosymbiosis between dinoflagellate algae of the family Symbiodiniaceae and marine invertebrates has powered coral reef productivity for millions of years. However, anthropogenic climate change, ocean acidification, and eutrophication are threatening this essential reef-building relationship. Considering these threats, it is crucial we understand how marine photosymbiosis functions and will likely change throughout the Anthropocene. While strides have been taken to understand photosymbiosis in endosymbiotic systems (symbionts living within host cells), such as corals, research into Fraginae cockles, a subfamily of exosymbiotic (symbionts living outside of host cells) marine bivalves, is lacking. To expand our understanding of marine photosymbiosis, this study investigates the effects of light on nitrogen assimilation and photosynthesis, two essential photosymbiotic pathways, in the *Cladocopium* symbionts inhabiting two species of Fraginae cockles, *Fragum sueziense* and *Fragum whitleyi.* The *Cladocopium* transcriptomes of *F. sueziense* (*Cladocopium SU*) and *F. whitleyi* (*Cladocopium WH*) exposed to light, dim, and dark conditions were collected and searched for differential expression across treatments. Eighty-two differentially expressed gene sequences (DEGs) representing seven photosynthesis protein complexes and five nitrogen assimilation enzymes were discovered. Overall, *Cladocopium SU* showed a greater number of photosynthesis DEGs while slightly more nitrogen assimilation DEGs were found in *Cladocopium WH*. These findings are the first look into the effects of environmental stress on the functioning of photosymbiotic pathways in *Cladocopium* inhabiting Fraginae cockles. With this study being the first of its kind on the *Cladocopium*-Fraginae system, it provides knowledge on sensitive photosynthesis and nitrogen assimilation enzymes/proteins which can act as the foundation of future research into the impacts of anthropogenic stressors on exosymbiosis.

Introduction

Coral reefs are typically located in oligotrophic, or nutrient-poor, environments (Roth 2014). Paradoxically, they are one of the world's most productive and biologically diverse ecosystems. Despite covering between 0.1% and 0.5% of the ocean floor (Moberg and Folke 1999), approximately 32% of all named marine species occur on coral reefs (Fisher et al. 2015). The paradoxical productivity of coral reefs can be explained by a photosymbiotic relationship between photosynthetic, dinoflagellate algae from the family Symbiodiniaceae and a variety of marine invertebrate hosts, including bivalves from the subfamily Fraginae (Family: Cardiidae).

Fraginae cockles and their resident Symbiodiniaceae algae exchange essential nutrients and create a thriving microcosm in which both parties' energy demands are met (Li et al. 2018). This mutually beneficial relationship is complex, constantly in flux, and dependent on a variety of biotic and abiotic factors. Among the most important of these factors is light, the most abundant resource in nutrient-limited coral reef environments. Light powers photosynthesis, the metabolic process responsible for meeting the energy demands of both host and symbiont. Energy from photosynthesis in part goes to nitrogen assimilation, a second essential photosymbiotic pathway (Chitnis 1996). The nitrogen assimilation pathway makes the most of limited nitrogen resources by recycling nitrogen between host and symbiont (Pernice et al. 2012). Processed nitrogen goes on to be an important component of photosynthetic functioning, creating a loop in which photosynthesis is reliant on nitrogen assimilation and vice versa. While the effects of light availability and other environmental stressors on the functioning of photosynthesis and nitrogen assimilation have been previously studied in corals and a handful of other photosymbiotic marine invertebrates, the Fraginae system is yet to be investigated.

Fraginae cockles are an excellent candidate for photosymbiotic research because they exhibit a relationship with algae distinctly different from that found in most well-studied photosymbiotic systems, such as corals. Corals are endosymbiotic. In endosymbiosis, symbionts reside within the gastrodermal cells of their host (Xiang et al. 2020). Contrarily, Fraginae cockles are exosymbiotic, wherein symbionts reside outside of host cells in tubule extensions of the digestive system (Farmer et al. 2001; Li et al. 2018). Additionally, Fraginae cockles have been found to occupy varying habitat types with different light availabilities. Contrasts within the Fraginae subfamily and between other photosymbiotic families will provide new perspectives on the many ways photosymbiosis functions in reef organisms.

Anthropogenic climate change (Hoegh-Guldberg 1999), ocean acidification (Hoegh-Guldberg et al. 2007), and nutrient enrichment (Zhao et al. 2021) are quickly changing the composition of coral reefs and disrupting the functioning of photosymbiotic relationships. Considering these threats, it is of utmost importance to have a detailed and inclusive understanding of photosymbiosis and its response to environmental change. In this thesis, I will advance our understanding of photosymbiosis by investigating the transcriptomic response of photosynthesis and nitrogen assimilation to changes in light availability in the *Cladocopium* algal inhabiting Fraginae cockles.

Study System

Of the 10 genera in the Fraginae subfamily, three exhibit obligate photosymbiosis (Kirkendale 2009). This study will focus on two species within the photosymbiotic genera *Fragum, Fragum sueziense* and *Fragum whitleyi.* The most abundant symbiont in *F. sueziense* and *F. whitleyi* is Symbiodiniaceae clade C, or *Cladocopium* (Li et al. 2018). *Cladocopium* is the

most species-rich and abundant genus within the family Symbiodiniaceae and is associated with a variety of marine invertebrate hosts aside from *F. sueziense* and *F. whitleyi* (LaJeunesse et al. 2018). Like all photosymbiotic Fraginae, *F. sueziense* and *F. whitleyi* possess special morphologies for hosting their *Cladocopium* symbionts, including an expanded symbiont-bearing mantle (Kirkendale 2009; Vermeij 2013). While both Fraginae species possess features adapted for photosymbiosis, they differ in their ecological niches within the Indo-Pacific reef habitats they occupy. *F. sueziense* live in sediment at a depth of five meters below the sea surface whereas *F. whitleyi* occupy sediment shallower than 0.5 meters. Experimentally manipulating Fraginae with different habitat depths allows for a comparison of response to light reduction between *Cladocopium* adjusted to different light availabilities.

Photosynthesis

The success of Fraginae-*Cladocopium* photosymbiosis depends on the ability of *Cladocopium* to create energy through photosynthesis. Photosynthesis, depicted in Figure 1, consists of two distinct processes: the light reactions and carbon fixation. The light reactions, and photosynthesis in general, begin with the harvesting of light by light-reaction antenna proteins (Bag 2021). Following, Photosystem II (PSII) uses this harvested light energy to split water and create electrons (Barber 2003). The cytochrome b6/f complex then transfers these electrons to Photosystem I (PSI) (Malone et al. 2019), where they are energized (Chitnis 1996). Finally, the energized electrons are transferred to the second photosynthetic process, carbon fixation, by the photosynthetic electron transport system (Moolna and Bowsher 2010). The transfer of electrons from PSII, to the cytochrome b6/f complex, to PSI creates a proton gradient which powers the creation of ATP by ATP synthase (Chitnis 1996). Energy created by PSI, PSII, and ATP

synthase is also sent to the carbon fixation process. Carbon fixation uses this energy, electrons from the photosynthetic electron transport system, and carbon dioxide to create organic compounds capable of powering cellular processes (Rochaix 2004). Carbon fixation is almost entirely catalyzed by the Rubisco enzyme (Paul 1996), but is upregulated by the catalytic enzyme fructose 1,6-biphosphate aldolase (Uematsu et al. 2012). Antenna proteins, PSII, cytochrome b6/f complex, PSI, ATP synthase, electron transporters, and carbon fixation are all essential to photosynthetic functioning and will be a focal point of this study. Table 1 summarizes the functions of these pathways.

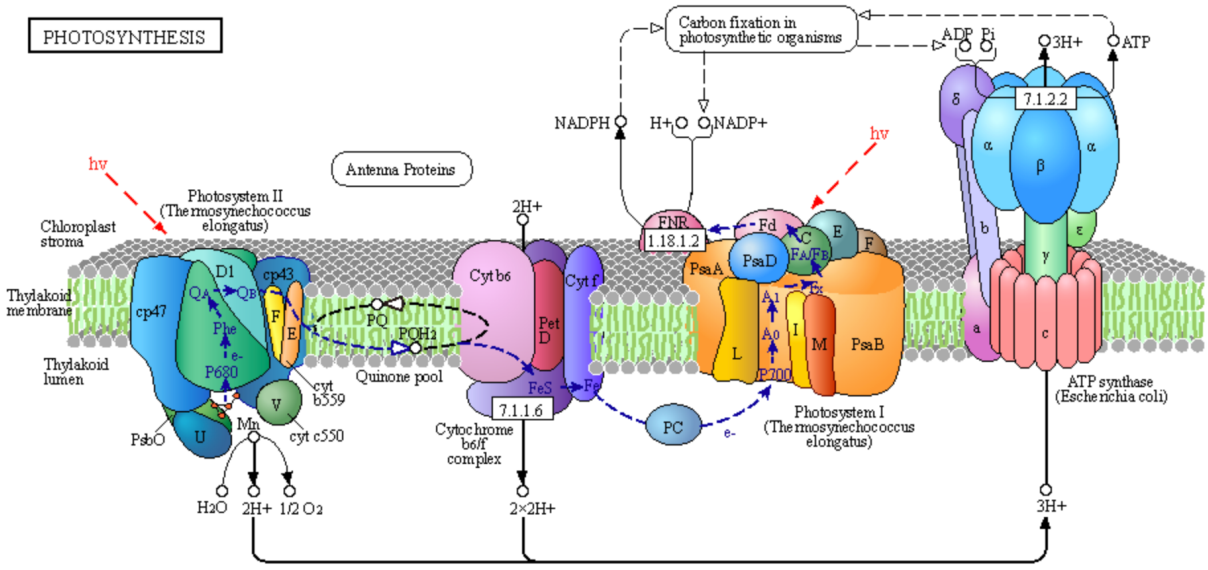

Figure 1: The protein complexes of photosynthesis. This study will analyze the transcriptomic response of enzymes/proteins from the major protein complexes shown above, including antenna proteins, PSII, cytochrome b6/f complex, PSI, electron transporters, ATP synthase, and carbon fixation. Retrieved from the Kyoto Encyclopedia of Genes and Genomes.

For photosynthesis to function as outlined above, *Cladocopium* symbionts must have a consistent source of light. Light absorbance by water and an increase in particulate matter at deeper depths has led to coral reefs to be predominantly located in shallow waters (< 30 meters), where light availability is highest (Roth 2014). Additionally, it has been found that symbionts

have developed a host of adaptations to maximize their photosynthetic efficiency. Among the most important of these adaptations is the cyclical nature of Symbiodiniaceae metabolic activities. The production of oxygen via photosynthesis is prioritized during the day while other metabolic processes which require energy are prioritized at night (Roth 2014). Symbiodiniaceae are also capable of dissipating light when under too high of illumination (Gorbunov et al. 2001), repairing daily damage caused by photosynthesis (Gorbunov et al. 2001), and increasing light-absorbing pigments when under low-light conditions (Falkowski and Dubinsky 1981). While Symbiodiniaceae algal have previously been found to have mechanisms for maximizing photosynthetic efficiency, this study will be the first to investigate the adaptability of *Cladocopium* inhabiting Fraginae cockles to their light environment.

Nitrogen Assimilation

This study is the first look into the transcriptomic response of photosynthesis to changes in light availability in *Cladocopium* inhabiting Fraginae cockles. However, this is not the full story. The success of photosymbiosis is also highly dependent on nutrient availability, particularly nitrogen. Nitrogen is the most-limiting nutrient in the low-latitude oceans which coral reefs occupy (Moore et al. 2013) and is a determining factor of Symbiodiniaceae growth and density (Rädecker et al. 2015). It is yet to be completely understood how the various genes involved in nitrogen assimilation function and act in concordance with one another. The metabolic integration of host and symbiont in photosymbiotic reef species further complicates the understanding we do have. However, recent transcriptomic research into photosymbiotic marine invertebrates has begun to piece together this confusing puzzle.

It is understood that both marine invertebrate hosts and their corresponding symbiont are capable of assimilating nitrogen independently (Rädecker et al. 2015; Morris et al. 2019). However, Symbiodiniaceae more readily uptake and metabolize nitrogen than their host (Pernice et al. 2012). Symbiodiniaceae are also more nitrogen limited than their host (Pernice et al. 2012). Nitrogen as a limiting factor for Symbiodiniaceae creates a feedback loop in which symbionts are forced to cap their own reproduction and translocate more photosynthates to their host cells (Morris et al. 2019). The benefits of nitrogen limitation incentivizes photosymbiotic hosts to regulate the amount of nitrogen available to their symbionts by promoting nitrogen delivery during the day and restricting it at night (Thies et al. 2021). Regulating nitrogen delivery ensures symbionts have the necessary nutrition to photosynthesize but do not over-populate. The benefit of nitrogen limitation has also led to the development of specialized pathways dedicated to the recycling of nitrogen between host and symbiont (Cui et al. 2019).

Among the pathways essential to the recycling and assimilation of nitrogen in photosymbiotic organisms is the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) cycle. Symbiodiniaceae GS/GOGAT enzymes assimilate ammonium to create amino acid products. These products are translocated to host cells to be used in photosynthesis and other cellular functions (Avila-Magaña 2020). GS/GOGAT also facilitate the recycling of nitrogen throughout the cytoplasm of endosymbiotic reef species (Pernice et al. 2012; Xiang et al. 2020). Finally, GS/GOGAT integrate nitrogen assimilation and photosynthesis by synthesizing glutamate, a metabolic precursor in chlorophyll biosynthesis (Avila-Magaña 2020). For GS/GOGAT to function, nitrogen must first be taken up by ammonium transporters in the plasma membrane (Howitt and Udvardi 2000). While Symbiodiniaceae algal most commonly uptake and assimilate nitrogen in the form of ammonium (Rädecker et al. 2015; DiRoberts et al.

2021), nitrogen can also be taken up as nitrite, nitrate, and host-derived urea (D'Elia et al. 1983; Grover et al. 2003; Grover et al. 2006; Ip et al. 2020). Nitrite reductase and urease can then degrade nitrite and urea into the more readily assimilated ammonium (Bouchard and Yamasaki 2008; Ip et al. 2020). This thesis will be the first to investigate the response of GS, GOGAT, ammonium transporters, nitrite reductase, and urease to changes in light availability in *Cladocopium* algal inhabiting Fraginae cockles. Figure 2 depicts these processes in an endosymbiotic coral cell and Table 1 summarizes their functions.

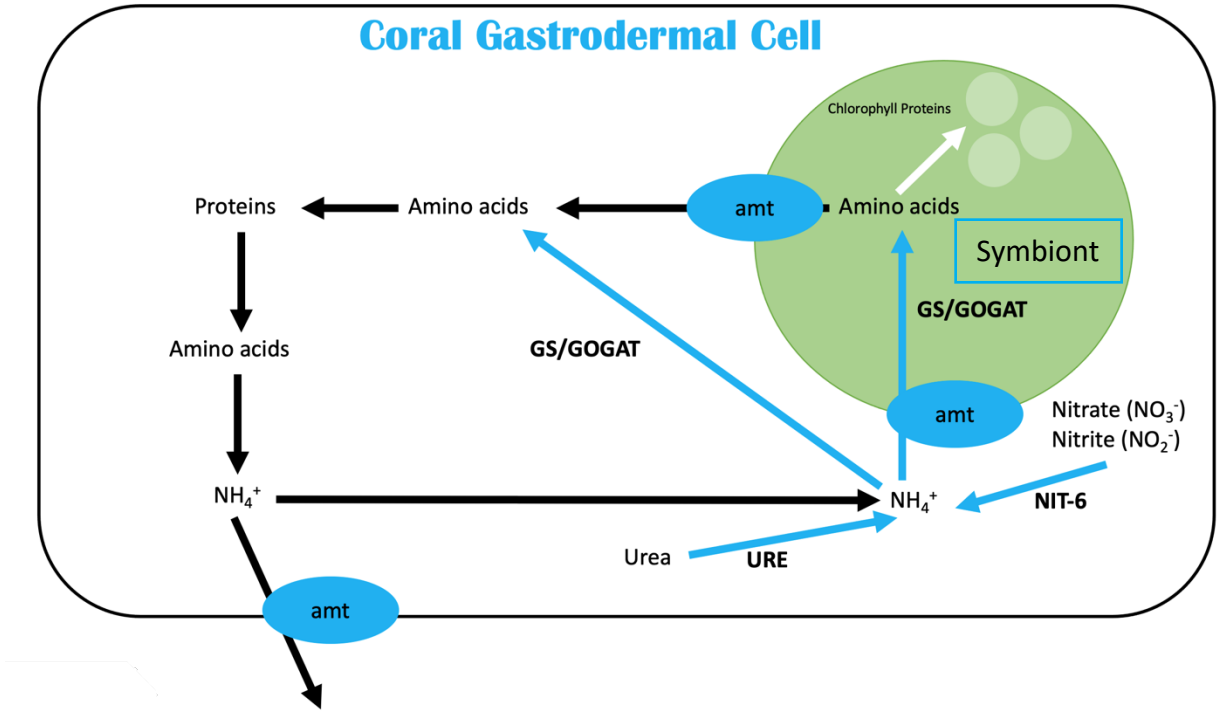

Figure 2: Nitrogen assimilation and recycling in a photosymbiotic coral cell. Important processes to this study are highlighted in blue and include ammonium transporters (amt), GS/GOGAT, urease (URE), and nitrite reductase (NIT-6). Figure adapted from Pernice et al. 2012 and Avila-Magaña 2020.

Research Overview

This thesis aims to understand (a) how the photosynthesis and nitrogen assimilation pathways of *Cladocopium* algal inhabiting *F. sueziense* (*Cladocopium SU*) and *Cladocopium* algal inhabiting *F. whitleyi* (*Cladocopium WH*) respond to changes in light, and (b) whether the

photosynthesis and nitrogen assimilation responses of *Cladocopium SU* and *Cladocopium WH* differ.

To come to these understandings, I will utilize the power of comparative transcriptomics, a method which allows for the understanding of the genetic basis of organismal response to stressors (DeBiasse and Kelly 2016). The transcriptomes of *Cladocopium SU* and *Cladocopium WH* will be searched for differential expression of the photosynthesis and nitrogen assimilation protein complexes/enzymes outlined in Table 1 between light, dim, and dark treatments. This method will pinpoint the precise gene sequences coding for photosynthesis and nitrogen assimilation which show a significant response to changes in light availability.

Photosynthesis	
Protein Complex	Function
Antenna Proteins	Traps light energy
Photosystem II (PSII)	Uses trapped light energy to split water and create free electrons
Cytochrome b6/f complex	Transfers electrons from PSII to PSI
Photosystem I (PSI)	Uses light energy to increase the energy of electrons created by PSII
Electron Transport	Last step of linear electron flow; transfers electrons to carbon fixation
ATP Synthase	Produces ATP using the proton gradient created by the electron transport chain of PSI, cytochrome b6/f complex, and PSII
Carbon Fixation	Uses energy from PSI, PSII, and ATP Synthase to create cellular energy from carbon dioxide
Nitrogen Assimilation	
Enzyme	Function
GS/GOGAT	Recycles nitrogen between symbiont and host; assimilates ammonium; synthesizes glutamate
Ammonium Transporters	Uptakes ammonium
Nitrite Reductase	Degrades nitrite into ammonium
Urease	Degrades urea into ammonium

Table 1: Photosynthesis and nitrogen assimilation protein complexes/enzymes of interest for this thesis.

Methods

Experimental Approach

F. whitleyi samples were collected from the Indo-Pacific basin in East Agana Bay, Guam (13°29'23.3"N 144°46'23.5"E) at a depth of less than 0.5 meters. *F. sueziense* samples were collected from Family Beach, Guam (13°27'46.7"N 144°38'48.7"E) at a depth of approximately 5 meters. All cockle samples were then transported to the University of Guam where tank light experiments were performed. Two tanks were supplied with running water from a reef lagoon and sand from the collection sites and shaded with mesh screens to reduce light intensity to that of the respective collection depths (663±233 μmol quanta m^{-2} s^{-1} for the *F. whitleyi* tank and 430±128 μmol quanta m^{-2} s^{-1} for the *F. sueziense* tank). Samples were left to acclimate to tank conditions for three days with constant light and temperature (30°C).

After a three-day acclimation period, the cockle specimens were divided into three tanks with differing light intensities, one control, one reduced light, and one darkness, and left for three days. *F. whitleyi* had 8 specimens placed in the control condition (663±233 μmol quanta m^{-2} s^{-1}, 30.8±0.16°C), 3 specimens in the light reduced, or dim, condition (210±54 μmol quanta m^{-2} s^{-1}, 30.8±0.16°C), and 7 specimens in the darkness condition (30.53±0.05°C). *F. sueziense* had 11 specimens in the control condition (430±128 μmol quanta m^{-2} s^{-1}, 30.76±0.12°C), 8 specimens in the dim condition (150±8 μmol quanta m^{-2} s^{-1}, 30.76±0.21°C), and 11 specimens in the darkness condition (30.5±0.08°C).

Following treatment, all specimens were immediately dissected. Collected tissues were preserved and sent to the Li Lab at University of Colorado at Boulder. RNA was extracted from the symbiont-bearing cockle tissues, checked for quality, and sequenced by members of the Li Lab. Transcriptome analyses, including assembly, quantification, and photosymbiont

transcriptome identification and separation were performed by Viridiana Avila-Magaña. These methods resulted in the assemblage of complete transcriptomes for the *Cladocopium* symbionts inhabiting *F. sueziense* (*Cladocopium SU*) and *F. whitleyi* (*Cladocopium WH*). Full protocol is described in a manuscript under preparation (Avila-Magaña et al. *in preparation*).

Gene Collection

KEGG IDs, identifiers of biological functions, for photosynthesis and nitrogen assimilation proteins/enzymes were collected using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). Based on previous photosymbiotic research and the pathways detailed on KEGG, 18 KEGG IDs of interest were identified for photosynthesis (Gierz et al. 2017) (Table 2) and 15 KEGG IDs of interest were identified for nitrogen assimilation (Pernice et al. 2012; Ip et al. 2020; Xiang et al. 2020) (Table 3). The photosynthesis and nitrogen assimilation KEGG IDs were then stored in separate lists in the Unix program.

Photosynthesis		
Pathway	Protein/Transcript	KEGG IDs
Light Reaction Antenna Proteins	*LHCA1*	K08907
Photosystem II (PSII)	*psbC, psbF, psbK, psbH, psbO, psbP*	K02705, K02708, K02712, K02709, K02716, K02717
Cytochrome b6/f complex	*petC*	K02636
Photosystem I (PSI)	*psaA, psaB, psaC, psaD, psaF, psaJ*	K02689, K02690, K02691, K02692, K02694, K02697
Photosynthetic Electron Transport	*petH*	K02641
ATP Synthase	*ATPF1G*	K02115
Carbon Fixation	*fbaA, rbcl*	K01624, K01601

Table 2: Photosynthesis KEGG IDs searched for gene expression in the *Cladocopium SU* and *Cladocopium WH* transcriptomes.

Nitrogen Assimilation		
Enzyme	Acronym	KEGG IDs
Ammonium Transporters	*amt*	K03320, K07573, K06580
Glutamine Synthetase	*GS*	K01915, K20712, K01949
Glutamate Synthase	*GOGAT*	K00264, K00265, K00266, K00284, K22083, K17792
Nitrite Reductase	*NIT-6*	K17877
Urease	*URE*	K01427, K01428

Table 3: Nitrogen assimilation KEGG IDs searched for gene expression in the *Cladocopium SU* and *Cladocopium WH* transcriptomes.

Following KEGG ID collection, the assembled *Cladocopium SU* and *Cladocopium WH* transcriptomes were annotated with the Emapper program (https://github.com/eggnogdb/eggnog-mapper/blob/master/emapper.py). Emapper detailed the ortholog score, best associated taxonomic level, KEGG ID, and KEGG pathway of every transcript, labeled as TRINITY IDs, in the complete *Cladocopium* transcriptomes. Using Unix, the previously compiled KEGG IDs were searched for within the *Cladocopium SU* and *Cladocopium WH* annotated transcriptomes. The KEGG ID search revealed all annotated transcripts coding for the nitrogen assimilation and photosynthesis enzymes/proteins of interest.

A process was then undergone to ensure all KEGG IDs of interest were annotated in Emapper and found in our search. The KEGG IDs of transcripts found from the Emapper search were compared to the full KEGG ID list using the program Venny (https://bioinfogp.cnb.csic.es/tools/venny/). A handful of KEGG IDs were not found in the transcripts resulting from the Emapper annotation and search. Transcripts associated with these KEGG IDs were manually found.

To begin, the missing KEGG IDs were searched for in the KEGG database. The gene sequences associated with these KEGG IDs were collected and stored in Unix. The manually selected gene sequences were collected from single-celled photosynthetic organisms closely

related to *Cladocopium,* including *Emiliania huxleyi* and *Chlamydomonas reinhardtii,* or the well-researched plant species *Arabidopsis thaliana,* depending on database availability.

The gene sequences collected from KEGG were compared against the *Cladocopium SU* and *Cladocopium WH* transcriptomes using the tBLASTn function of the Basic Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Like Emapper, the tBLASTn search identified transcripts, labeled as TRINITY IDs, within the *Cladocopium* transcriptomes coding the enzymatic functions associated with the compiled photosynthesis and nitrogen assimilation KEGG IDs. These transcripts were checked for significant alignment against the NCBI amino acid database using BlastX. Any transcripts found to be associated with bacteria or with a bivalve were removed, ensuring all transcripts were from the photosymbiont. For ease of further analysis, the transcripts occurring from the Emapper annotation and the manual BLAST were compiled into four lists: *Cladocopium WH* nitrogen assimilation, *Cladocopium WH* photosynthesis, *Cladocopium SU* nitrogen assimilation, and *Cladocopium SU* photosynthesis.

DEG Determination

Using the Unix software, the four lists of TRINITY IDs were separately searched for in the *Cladocopium* gene expression matrices of their respective species. The gene expression matrices consist of all gene sequences and their associated transcription, normalized as transcripts per million (TPM), across the three experimental light conditions and their respective replicates. These matrices were previously obtained by Viridiana Avila-Magaña using transcriptome standard pipelines, such as Trinity (cit) (Avila-Magaña et al. *in preparation*). The expression of the previously compiled TRINITY IDs across all conditions and replicates resulted

from the gene expression matrix search. This data was then moved into Excel, with each TRINITY ID list placed into a separate sheet for ease of data filtration.

Data filtration began with the removal of minimally expressed transcripts. Specifically, genes with less than 10 TPM of expression in at least one condition or replicate were removed, as it has been done in coral-algal photosymbiosis, and other transcriptomic studies (Selvaraju et al. 2017; Summers et al. 2020; Avila-Magaña 2020; Dubois et al. 2021). T-tests were performed on the remaining transcripts to find significantly differentially expressed genes (DEGs) across the light, dim, and dark treatments as performed in a previous photosymbiosis study (Xiang et al. 2020). The previous pipeline allows for the comparison of differential expression within a single gene in one species across different light treatments. Transcripts with a significant t-test (p-value ≤ 0.05) between the light and dark conditions (LDk), light and dim conditions (LD), and/or dim and dark conditions (DDk) were separated out and determined to be DEGs. Finally, the average expression of the replicates within the light, dim, and dark conditions was calculated for each DEG.

R Studio (version 1.2.158) was used to create boxplots and slope charts to depict trends in gene transcription. Boxplots, created using ggplot (https://ggplot2.tidyverse.org/reference/ggplot.html), compare the log transformed average transcription of nitrogen assimilation and photosynthesis enzymes across light, dim, and dark conditions within species. Additional t-tests were run in Excel to determine if nitrogen assimilation and photosynthesis average transcription significantly changed across conditions. Slope charts, created using ggplot, compared the average transcriptions of individual DEGs across all three conditions within species.

Results

Gene Collection

In total, 143 transcripts were found to code for the photosynthesis and nitrogen assimilation KEGG IDs of interest (Tables 2 and 3) in *Cladocopium SU* and 511 were found in *Cladocopium WH.* Of the 143 *Cladocopium SU* transcripts, 49 were found to have greater than 10 TPM in at least one replicate and a significant difference in expression (p-value < 0.05) in one or more light-dark (LDk), light-dim (LD), and dim-dark (DDk) comparison. Of the 511 *Cladocopium WH* coding transcripts, 33 had at least one replicate with greater than 10 TPM and a significant difference in at least one LDk, LD, and DDk comparison. Table 4 shows the complete breakdown of transcript and DEG collection in *Cladocopium SU* and *Cladocopium WH*.

	Cladocopium SU		*Cladocopium WH*	
	Photosynthesis	Nitrogen Assimilation	Photosynthesis	Nitrogen Assimilation
Total # of transcripts	83	60	260	251
# LDk DEGs	22	19	9	20
# LD DEGs	10	12	1	3
# DDk DEGs	13	11	1	3
Total # of DEGs	**29**	**20**	**10**	**23**
Species DEG total		**49**		**33**

Table 4: Number of photosynthesis and nitrogen assimilation transcripts and DEGs found in the transcriptomes of *Cladocopium SU* and *Cladocopium WH.*

Cladocopium SU has a total of 29 photosynthesis DEGs and *Cladocopium WH* has a total of 10 photosynthesis DEGs. Table 5 shows the distribution of these DEGs amongst the seven investigated photosynthesis protein complexes. *Cladocopium SU* has DEGs in all seven protein complexes while *Cladocopium WH* has DEGs in five of the seven protein complexes.

Additionally, *Cladocopium SU* has a greater abundance of DEGs than *Cladocopium WH* in all protein complexes except photosynthetic electron transport.

Cladocopium SU was also found to have 20 nitrogen assimilation DEGs while *Cladocopium WH* revealed 23 nitrogen assimilation DEGs. The distribution of these DEGs amongst the five nitrogen assimilation enzymes investigated in this study is seen in Table 5. *Cladocopium WH* shows DEGs in all five enzymes while *Cladocopium SU* shows DEGs in all but the *URE* enzyme.

Photosynthesis		**Nitrogen Assimilation**	
Light Reaction Antenna Proteins		***NIT-6***	
Cladocopium SU	1	*Cladocopium SU*	1
Cladocopium WH	0	*Cladocopium WH*	2
PSII		***URE***	
Cladocopium SU	9	*Cladocopium SU*	0
Cladocopium WH	4	*Cladocopium WH*	1
Cytochrome b6/f complex		***amt***	
Cladocopium SU	1	*Cladocopium SU*	11
Cladocopium WH	0	*Cladocopium WH*	7
PSI		***GS***	
Cladocopium SU	7	*Cladocopium SU*	7
Cladocopium WH	1	*Cladocopium WH*	9
Photosynthetic Electron Transport		***GOGAT***	
Cladocopium SU	1	*Cladocopium SU*	1
Cladocopium WH	2	*Cladocopium WH*	4
ATP Synthase			
Cladocopium SU	2		
Cladocopium WH	0		
Carbon Fixation			
Cladocopium SU	8		
Cladocopium WH	3		

Table 5: Distribution of *Cladocopium SU* and *Cladocopium WH* DEGs amongst the investigated photosynthesis protein complexes and nitrogen metabolism enzymes. Colored bars show the difference in DEG amounts between *Cladocopium* variants in each protein complex/enzyme.

Cladocopium SU **DEGs**

Figure 3 depicts the log transformed average transcription of nitrogen assimilation, photosynthesis light reactions (light reaction antenna proteins, PSII, cytochrome b6/f complex,

PSI, photosynthetic electron transport, and ATP synthase), and carbon fixation in *Cladocopium SU* in light, dim, and dark conditions. Points represent the average transcription of individual DEGs. Nitrogen assimilation shows a trend of downregulation across conditions, but this trend is not statistically significant. Photosynthesis light reactions also exhibit downregulation across conditions and have a significant difference in expression in the LDk comparison (p-value: 0.028667). Carbon fixation shows LD downregulation and DDk upregulation, but neither of these changes are statistically significant.

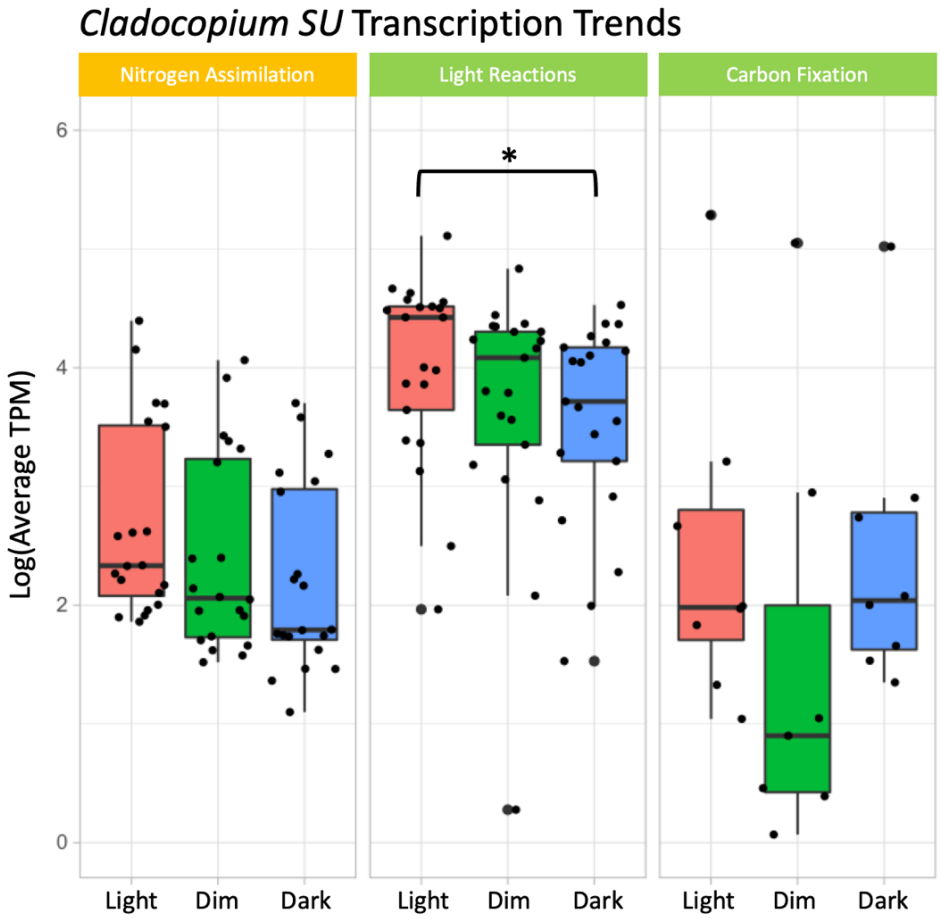

Figure 3: Log transformed average transcription of nitrogen assimilation, photosynthesis light reactions, and carbon fixation DEGs across light, dim, and dark conditions in *Cladocopium SU.* Points are representative of the average transcription of individual DEGs. Significant changes are marked with an asterisk.

The average trends depicted in Figure 3 encompass 49 DEGs with at least one significant condition comparison (LDk, LD, Ddk) (Table 6). These DEGs represent all enzymes/protein complexes investigated except for the nitrogen assimilation enzyme *URE*. Figure 4 depicts the transcription trends of all 49 DEGs in the light, dim, and dark conditions. All occurring enzymes associated with nitrogen assimilation, including *amt*, *GOGAT*, *GS*, and *NIT-6* exhibit genes with LDk and LD significant downregulation. All light reaction proteins also exhibit DEGs with LDk or LD downregulation. Carbon fixation shows a variation in pattern between *fbaA* and *rbcl.* *FbaA* has LDk and LD downregulation while *rbcl* only exhibits DDk significant upregulation in expression.

Cladocopium SU									
Transcript	Enzyme	Function	Kegg ID	Light Average (n = 11)	Dim Average (n = 8)	Dark Average (n = 11)	LDk P-Value	LD P-Value	DDk P-Value
TRINITY_DN232052_c0_g1	amt	Nitrogen Assimilation	ko:K06580	9.1454	7.0480	5.8109	0.00315 **		
TRINITY_DN128755_c0_g1	amt	Nitrogen Assimilation	ko:K03320	8.7599	5.5029	4.3256	0.00107 **	0.0131 *	
TRINITY_DN959371_c0_g1	amt	Nitrogen Assimilation	ko:K03320	7.0840	5.2602	3.0045	0.000152 ***	0.0413 *	0.000344 ***
TRINITY_DN253103_c0_g1	amt	Nitrogen Assimilation	ko:K03320	6.7780	5.6830	4.3186	0.000129 ***		0.00136 **
TRINITY_DN8040_c0_g2	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	13.6199	10.9482	8.7118	0.00767 **		
TRINITY_DN60266_c0_g4	amt	Nitrogen Assimilation	ko:K03320	7.4177	5.0570	5.7413	0.0471 *	0.0120 *	
TRINITY_DN1138313_c0_g1	amt	Nitrogen Assimilation	ko:K03320	9.6415	7.7590	5.7135	0.000734 ***		0.00689 **
TRINITY_DN6194_c0_g1	amt	Nitrogen Assimilation	ko:K03320	6.6768	4.5701	3.9134	0.00172 **	0.0117 *	
TRINITY_DN8040_c0_g1	amt	Nitrogen Assimilation	ko:K03320	10.3414	7.0837	5.6788	0.000554 ***	0.00386 **	
TRINITY_DN516107_c0_g1	amt	Nitrogen Assimilation	ko:K03320	63.6145	50.1497	40.5423	0.00154 **	0.0479 *	0.00774 **
TRINITY_DN690_c0_g1	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	6.4303	4.8417	6.0155		0.0298 *	
TRINITY_DN8359_c2_g1	GOGAT	Nitrogen Assimilation	ko:K00264	10.2809	8.5144	5.9865	0.000768 ***		0.00145 **
TRINITY_DN372_c0_g1	GS	Nitrogen Assimilation	ko:K01915	40.2812	29.4395	26.4417	0.0142 *		
TRINITY_DN670_c0_g3	GS	Nitrogen Assimilation	ko:K01915	33.2188	24.6189	19.2117	0.000900 ***	0.0275 *	0.00920 **
TRINITY_DN1138408_c0_g1	GS	Nitrogen Assimilation	ko:K01915	13.7585	11.0165	9.6040	0.00238 **	0.0357 *	0.0223 *
TRINITY_DN138409_c0_g1	GS	Nitrogen Assimilation	ko:K01915	8.1946	6.7548	5.0764	0.00235 **		0.00561 **
TRINITY_DN480_c0_g1	GS	Nitrogen Assimilation	ko:K01915,ko:K01949	13.2260	7.9231	9.1913	0.0176 *	0.00472 **	
TRINITY_DN55946_c0_g1	GS	Nitrogen Assimilation	ko:K01915	40.6007	30.8010	22.5627	0.000538 ***	0.0343 *	0.000629 ***
TRINITY_DN670_c0_g2	GS	Nitrogen Assimilation	ko:K01915	34.7189	27.6166	20.9852	0.00181 **		0.00101 **
TRINITY_DN9525_c0_g1	NIT-6	Nitrogen Assimilation	ko:.K17877	81.1290	58.2086	35.9494	0.000299 ***	0.0266 *	7.9E-5 ***
TRINITY_DN198096_c0_g1	ATPF1G	ATP Synthase	ko:K02115	38.2732	28.5454	24.8610	0.00181 **	0.0221 *	
TRINITY_DN198096_c0_g2	ATPF1G	ATP Synthase	ko:K02115	47.4635	36.4710	34.8252	0.0161 *	0.0377 *	
TRINITY_DN246189_c1_g1	rbcL	Carbon Fixation	ko:K01601	3.7770	1.0706	3.8565			0.0457 *
TRINITY_DN24047_c11_g3	rbcL	Carbon Fixation	ko:K01601	2.8360	0.3496	7.4058			0.0399 *
TRINITY_DN24047_c8_g4	rbcL	Carbon Fixation	ko:K01601	6.2522	2.8530	5.2443			0.0214 *
TRINITY_DN756857_c0_g1	rbcL	Carbon Fixation	ko:K01601	7.3293	1.4787	7.9821			0.0402 *
TRINITY_DN85898_c0_g1	rbcL	Carbon Fixation	ko:K01601	14.3951	2.4619	15.4715			0.0376 *
TRINITY_DN3115_c0_g3	fbaA	Carbon Fixation	ko:K01624	197.6613	156.0455	151.5619	0.0335 *		
TRINITY_DN371772_c0_g1	fbaA	Carbon Fixation	ko:K01624	7.1818	1.5818	4.6324			0.0268 *
TRINITY_DN3115_c0_g1	fbaA	Carbon Fixation	ko:K01624	24.7927	19.0957	18.2655	0.0163 *	0.0440 *	
TRINITY_DN15838_c0_g1	petC	Cytochrome b6-f complex	ko:K02636	83.4324	59.4069	57.7412	0.00977 **	0.0193 *	
TRINITY_DN823620_c1_g1	LHCA1	Antenna Proteins	ko:K08907	165.8484	125.7422	92.6557	0.000326 ***	0.0227 *	0.00188 **
TRINITY_DN5123_c0_g1	petH	Electron Transport	ko:K02641	53.4788	44.2313	41.0959	0.0346 *		
TRINITY_DN5861_c0_g1	psaA/psaB	PSI	ko:K02689,ko:K02690	29.6109	21.3123	7.3497	0.000739 ***		0.000747 ***
TRINITY_DN94241_c3_g1	psaC	PSI	ko:K02691	90.9303	77.2395	64.8206	0.00670 **		
TRINITY_DN353374_c0_g1	psaC	PSI	ko:K02691	106.2898	85.0539	79.1282	0.0268 *		
TRINITY_DN16805_c0_g1	psaD	PSI	ko:K02692	102.4561	79.0981	78.8493	0.0296 *	0.0426 *	
TRINITY_DN51876_c0_g1	psaF	PSI	ko:K02694	94.9879	73.8918	71.2152	0.0423 *		
TRINITY_DN67135_c0_g2	psaJ	PSI	ko:K02697	91.5023	73.9763	62.8029	0.00121 **		
TRINITY_DN67135_c0_g1	psaJ	PSI	ko:K02697	88.6455	68.4128	57.1538	0.00761 **		0.0131 *
TRINITY_DN55164_c0_g1	psbC	PSII	ko:K02705	96.8594	69.2434	26.6401	0.000802 ***		0.00429 **
TRINITY_DN4173_c0_g3	psbF	PSII	ko:K02708	83.5161	64.2439	60.5306	0.00312 **	0.00908 **	
TRINITY_DN4173_c0_g4	psbF	PSII	ko:K02708	90.1310	77.6397	67.5065	0.0172 *		
TRINITY_DN4173_c0_g2	psbF	PSII	ko:K02708	54.7787	44.8289	39.1922	0.00248 **	0.0315 *	
TRINITY_DN1091711_c0_g1	psbH	PSII	ko:K02709	28.9809	24.0904	18.4491	0.000302 ***		0.0471 *
TRINITY_DN263417_c0_g1	psbH	PSII	ko:K02709	7.1363	1.3198	9.7661			0.0473 *
TRINITY_DN1143131_c0_g1	psbP	PSII	ko:K02717	12.1582	8.0073	4.6143	0.000206 ***	0.0182 *	0.000246 ***
TRINITY_DN1047350_c0_g1	psbP	PSII	ko:K02717	22.8616	17.8763	15.0984	0.00134 **		
TRINITY_DN208278_c0_g1	psbP	PSII	ko:K02717	47.7709	35.2181	31.1718	0.000563 ***	0.00899 **	

Table 6: The 49 transcripts found to be DEGs in *Cladocopium SU*, including their associated function, KEGG ID, average expression in the three treatment groups, and significant p-values across condition comparisons.

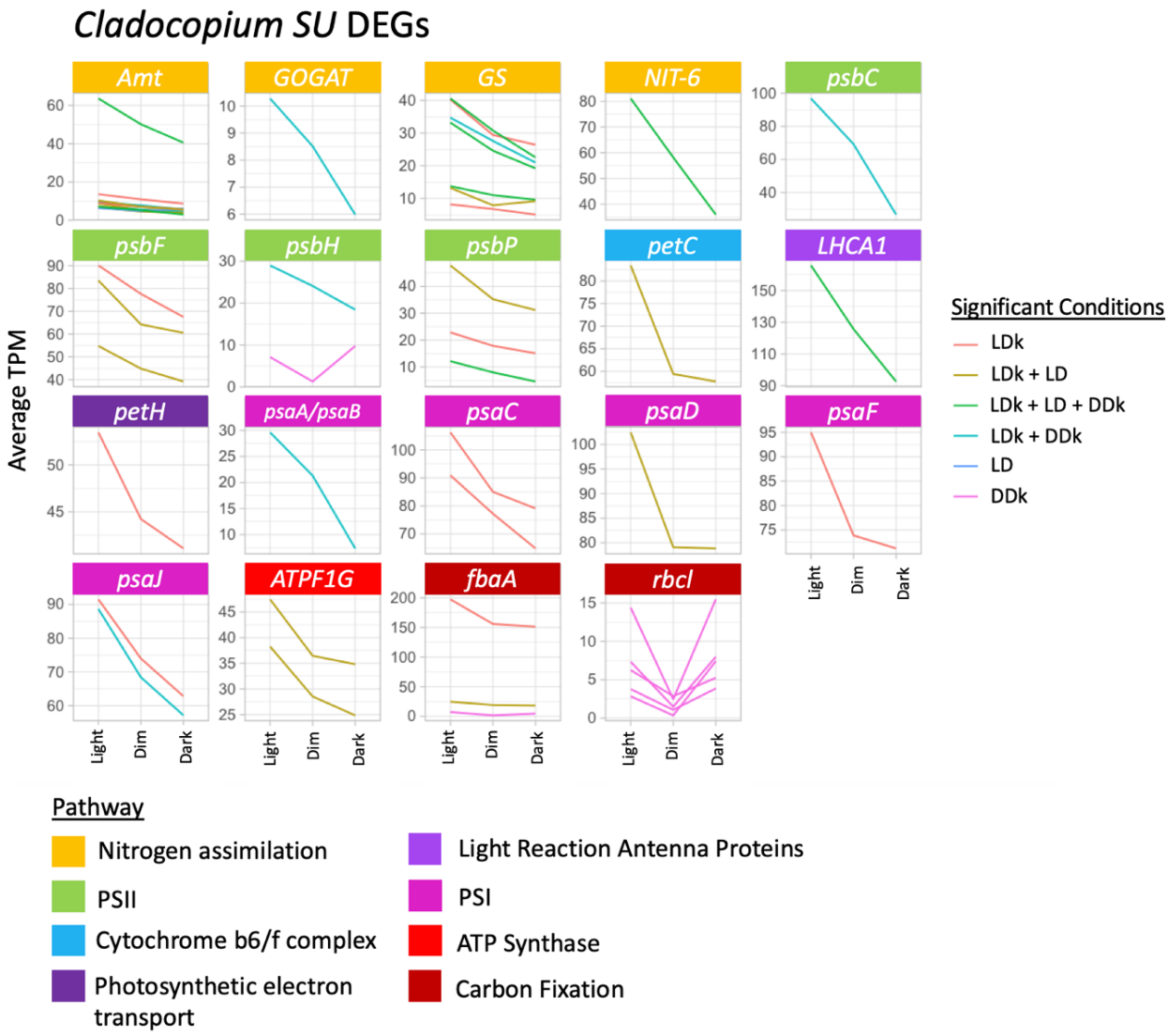

Figure 4: Average transcription of the 49 photosynthesis and nitrogen assimilation DEGs across treatments in *Cladocopium SU.* Each line is representative of an individual DEG with a significant difference in expression in at least one condition comparison (p-value ≤ 0.05, t-test). The color of each line corresponds to the change in conditions in which the significant difference in expression was observed.

Cladocopium WH symbiont DEGs

Figure 5 depicts the log transformed average transcription of nitrogen assimilation, photosynthesis light reactions, and carbon fixation in *Cladocopium WH* in light, dim, and dark conditions. Each point is representative of the average transcription of a single DEG. Nitrogen assimilation has significant LDk downregulation (p-value: 0.04031). Light reactions and carbon

fixation also show a trend of downregulation between light and dark conditions, but these trends are not statistically significant.

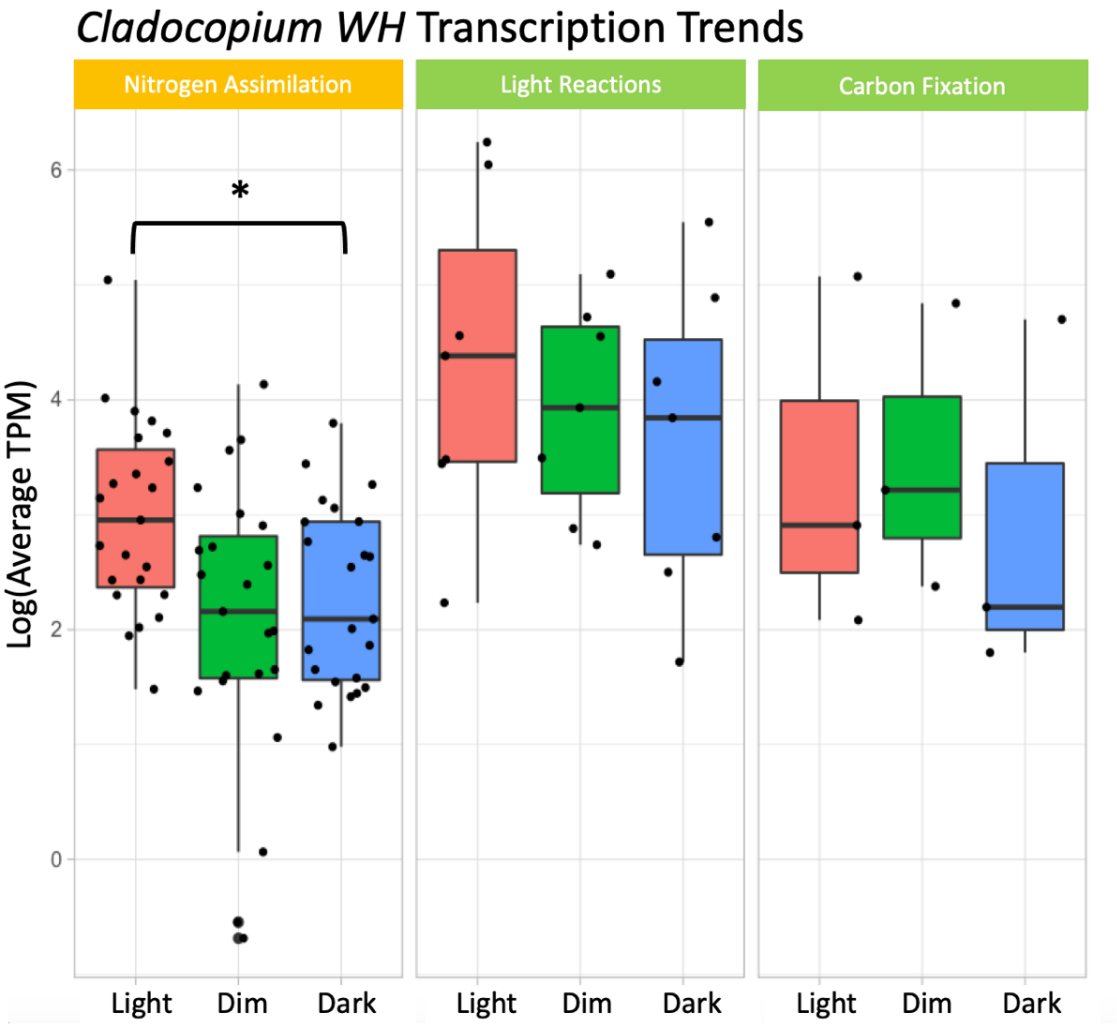

Figure 5: Log transformed average transcription of the DEGs related to nitrogen assimilation, photosynthesis light reactions, and carbon fixation, across light, dim, and dark treatments in *Cladocopium WH.* Points are representative of the average transcription of individual DEGs. Significant changes are marked with an asterisk.

33 DEGs are contained within the averages depicted in Figure 5 (Table 7). These DEGs represent all investigated enzymes associated with nitrogen assimilation and all protein complexes associated with photosynthesis except for the cytochrome b6/f complex and light reaction antenna proteins. Figure 6 depicts the average transcription of all 33 *Cladocopium WH*

DEGs across conditions. There is significant LDk downregulation in all nitrogen assimilation related enzymes, including *amt, GOGAT, GS, NIT-6*, and *URE*. Additionally, all photosynthesis related enzymes with a DEG occurring in *Cladocopium WH* exhibited transcripts with downregulation in the LDk and LD comparisons.

Cladocopium WH									
Transcript ID	Enzyme	Function	Kegg ID	Light Average (n = 8)	Dim Average (n = 3)	Dark Average (n = 7)	LDk P-Value	LD P-Value	DDk P-Value
TRINITY_DN79364_c1_g1	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	155.0807	62.5183	44.5900	0.0332 *		
TRINITY_DN9044_c1_g1	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	7.0061	4.3330	2.6656	0.0212 *		
TRINITY_DN15944_c0_g1	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	12.7699	5.2179	4.6897	0.00222 **		
TRINITY_DN36094_c0_g1	amt	Nitrogen Assimilation	ko:K03320	15.3465	7.3038	8.1103	0.0488 *		
TRINITY_DN3173_c0_g1	amt	Nitrogen Assimilation	ko:K03320	9.9901	8.6526	4.8524	0.0204 *		
TRINITY_DN36094_c1_g1	amt	Nitrogen Assimilation	ko:K03320	19.1850	12.9317	6.2018	0.0122 *		
TRINITY_DN28246_c1_g1	amt	Nitrogen Assimilation	ko:K03320,ko:K07573	40.9323	25.4296	18.9329	0.0404 *		
TRINITY_DN191064_c0_g1	GOGAT	Nitrogen Assimilation	ko:K00264	4.4010	0.5034	4.1228		0.00846 **	0.00698 **
TRINITY_DN26580_c0_g1	GOGAT	Nitrogen Assimilation	ko:K00264	28.6349	11.9233	14.1248	0.000212 ***		
TRINITY_DN356466_c0_g1	GOGAT	Nitrogen Assimilation	ko:K00264	23.2305	4.9574	12.7341	0.0476 *	0.0362 *	
TRINITY_DN102618_c0_g1	GOGAT	Nitrogen Assimilation	ko:K00266	7.5302	1.0675	7.4484		0.0103 *	0.0354 *
TRINITY_DN66415_c0_g1	GS	Nitrogen Assimilation	ko:K01915	10.0232	7.1713	4.2459	7.76E-6 ***		
TRINITY_DN28113_c2_g1	GS	Nitrogen Assimilation	ko:K01915	55.4228	35.2290	31.2754	0.00989 **		
TRINITY_DN147791_c0_g1	GS	Nitrogen Assimilation	ko:K01915	26.3416	14.7279	15.9022	0.00294 **		
TRINITY_DN30402_c0_g1	GS	Nitrogen Assimilation	ko:K01915	25.4029	18.2786	13.9543	0.00798 **		
TRINITY_DN116153_c0_g1	GS	Nitrogen Assimilation	ko:K01915	31.9698	20.2640	18.8773	0.00382 **		
TRINITY_DN8017_c0_g1	GS	Nitrogen Assimilation	ko:K01915	14.1574	10.9488	6.4487	0.0257 *		
TRINITY_DN24033_c0_g1	GS	Nitrogen Assimilation	ko:K01915	45.4443	15.1907	22.8181	0.00140 **		
TRINITY_DN21453_c0_g1	GS	Nitrogen Assimilation	ko:K01915	11.4090	4.7274	5.2190	0.000206 ***		
TRINITY_DN25425_c0_g2	GS	Nitrogen Assimilation	ko:K01915	49.4919	38.5745	21.2848	0.0471 *		
TRINITY_DN3726_c0_g1	NIT-6	Nitrogen Assimilation	ko:K17877	39.2545	0.5793	26.1390			0.0412 *
TRINITY_DN251093_c0_g2	NIT-6	Nitrogen Assimilation	ko:K17877	8.2107	2.8911	3.8297	0.0322 *		
TRINITY_DN47338_c0_g1	URE	Nitrogen Assimilation	ko:K01427	11.3755	5.0362	4.4641	0.000819 ***		
TRINITY_DN54893_c38_g1	rbcL	Carbon Fixation	ko:K01601	18.3326	24.9214	8.9875	0.0249 *		
TRINITY_DN178821_c0_g1	fbaA	Carbon Fixation	ko:K01624	8.0300	10.7719	6.0540			0.00220 **
TRINITY_DN30861_c0_g2	fbaA	Carbon Fixation	ko:K01624	159.8483	126.5559	110.0047	0.0131 *		
TRINITY_DN295858_c1_g2	petH	Electron Transport	ko:K02641	95.4977	94.9011	63.9902	0.0398 *		
TRINITY_DN43605_c0_g2	petH	Electron Transport	ko:K02641	80.1303	51.0338	46.6965	0.00153 **		
TRINITY_DN62613_c0_g1	psaA/psaB	PSI	ko:K02689,ko:K02690	422.9511	112.2514	132.8928	0.000846 ***	0.0163 *	
TRINITY_DN1013493_c2_g1	psbC	PSII	ko:K02705	514.7200	163.1125	256.4496	0.00195 **		
TRINITY_DN175677_c0_g1	psbH	PSII	ko:K02709	31.3186	32.9377	12.1903	0.00783 **		
TRINITY_DN61918_c0_g1	psbH	PSII	ko:K02709	9.3366	15.4716	5.5771	0.00742 **		
TRINITY_DN11713_c0_g1	psbP	PSII	ko:K02717	32.4833	17.8290	16.5149	1.64E-5 ***		

Table 7: 33 transcripts found to be DEGs in *Cladocopium WH*, including their associated function, KEGG ID, average expression in the three treatment groups, and significant p-values across conditions.

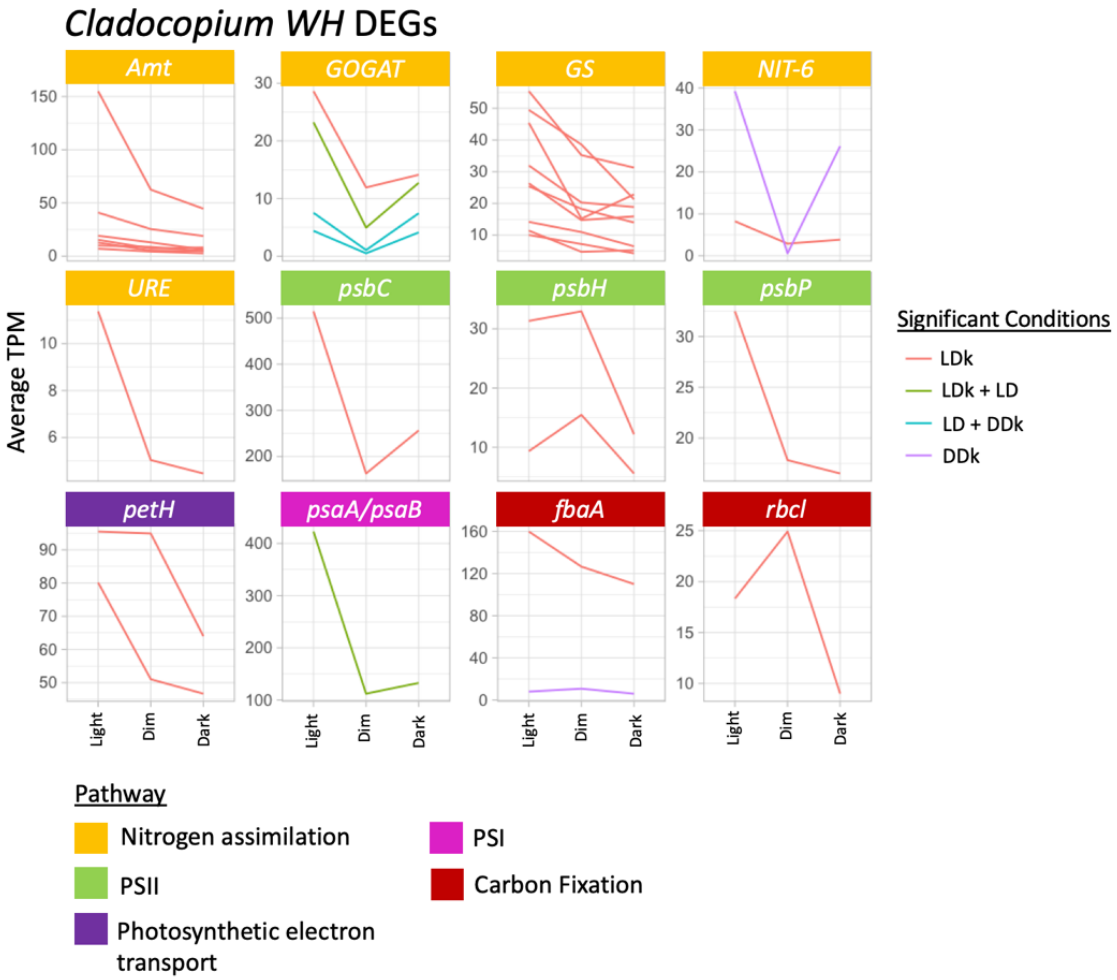

Figure 6: Average transcription of nitrogen assimilation and photosynthesis DEGs across treatments in *Cladocopium WH.* Each line is representative of an individual DEG with a significant change in at least one condition comparison (p-value ≤ 0.05, t-test). Line color corresponds to the condition comparisons in which each DEG showed a significant change.

Discussion

Downregulation of photosynthesis and nitrogen assimilation in dark conditions

Cladocopium SU and *Cladocopium WH* downregulate the light reactions of photosynthesis, including light reaction antenna proteins, PSII, cytochrome b6/f complex, PSI, electron transport, and ATP synthase, when exposed to dark conditions. These results are to be expected as light is the primary driver of photosynthesis. They are also findings consistent with

previous research on the effects of diel cycles on giant clam (genus *Tridacna*) photosymbionts. *Tridacna* symbionts downregulate the light reaction protein complex PSII at night (Liu et al. 2021). Like Fraginae cockles, *Tridacna* exhibit exosymbiosis in which Symbiodiniacae algae inhabit tubule extensions of the digestive system (Norton et al. 1992). However, *Tridacna* are like endosymbiotic systems with transparent symbiont-bearing tissues like corals, jellyfish, and anemones in that their symbionts are uncovered by shell and have easy access to light (Vermeij 2013). Photosymbiotic systems with high-light exposure can be adaptable to damaging irradiance conditions by downregulating their activity of PSI and PSII (Gorbunov et al. 2001; Jimenez et al. 2012). However, Fraginae cockles bury themselves in sediment and have shells covering their photosymbionts. The ecological niche of Fraginae and an adaptability of *Cladocopium* to low-light (Iglesias-Prieto and Trench 1994; Iglesias-Prieto and Trench 1997) suggests *Cladocopium SU* and *Cladocopium WH* may not have the capacity to photoacclimate under high-light conditions as they do for low-light conditions. Future research should expose *F. whitleyi* and *F. sueziense* to high-light and analyze the transcriptomic response of their extracellular *Cladocopium* to determine how their capacity for photoadaptation compares to *Tridacna* and endosymbiotic species.

Cladocopium SU and *Cladocopium WH* also exhibit downregulation of nitrogen assimilation when in dark conditions. Nitrogen assimilation activity in light-stressed photosymbiotic systems is yet to be investigated. However, it has been studied in Symbiodiniaceae exposed to nitrogen limitation and heat stress. Nitrogen assimilation activity, particularly GS/GOGAT, is downregulated in cultured Symbiodiniaceae under nitrogen limitation (Li et al. 2021) and in endosymbiotic Symbiodiniaceae in heat stress (Tang et al. 2020). The observed downregulation of nitrogen assimilation in *Cladocopium* exposed to dark

conditions contributes to the consensus that Symbiodiniaceae lose nitrogen assimilation function when under environmental stress.

In the present study, the downregulation of nitrogen assimilation in *Cladocopium SU* and *Cladocopium WH* in dark conditions may be closely tied to the downregulation of photosynthesis. Photosynthesis provides the necessary carbohydrates for nitrogen assimilation to synthesize amino acids (Yoneyama and Suzuki 2020) and nitrogen assimilation is a metabolic precursor to chlorophyll biosynthesis, an essential first step in photosynthesis (Avila-Magaña 2020). The link between these two pathways may create a negative feedback loop in which the downregulation of one pathway leads to the subsequent downregulation of the other. As carbohydrates and amino acids are depleted without efficient regeneration, the downregulation of photosynthesis and nitrogen assimilation compounds. *Cladocopium SU* and *Cladocopium WH* could potentially temporarily offset a negative feedback loop of photosynthesis and nitrogen assimilation downregulation by heterotrophically acquiring amino acids or assimilating nitrogen derived from the waste products of their Fraginae hosts (Martinez et al. 2022). The Fraginae host could also increase their own uptake and assimilation of nitrogen to supplement losses in symbiont-derived amino acids (Hemond and Vollmer 2015; Li et al. 2021; Cui et al. 2022). However, prolonged loss of nitrogen assimilation by *Cladocopium* will likely lead to an eventual breakdown of symbiosis (Rädecker et al. 2021).

Predictions can be made about the behavior of *Cladocopium*-Fraginae symbiosis following a loss of photosynthesis and nitrogen assimilation function, but it is still unknown how nutrients are exchanged and recycled when symbionts are not living within their host's cells. Future research may utilize a combination of isotope labeling technology and transcriptomics to track changes in the movement of nutrients throughout the *Cladocopium*-Fraginae system as

differential expression occurs under environmental stress. The combination of these technologies will broaden our understanding of nutrient exchange to the less studied exosymbiotic systems and help us to understand how photosynthesis and nitrogen assimilation work in tandem.

Transcriptional differences between *Cladocopium SU* and *Cladocopium WH*

Cladocopium SU and *Cladocopium WH* show similar trends in overall nitrogen assimilation and photosynthesis transcription, but a deeper look into each species' observed DEGs reveals a host of transcriptional differences. Most apparent of these differences is *Cladocopium SU* having a greater diversity of photosynthesis DEGs. The different ecological niches of *F. sueziense* and *F. whitleyi* may be an underlying factor contributing to this difference in photosynthetic response. *F. sueziense* lives at a greater depth (5 meters), and is adapted to lower average light conditions, than *F. whitleyi* (0.5 meters). Photosymbiotic corals inhabiting light-limited environments have greater chlorophyll content than high-light adapted corals (Falkowski and Dubinsky 1981) and endosymbiotic Symbiodiniaceae show increased photosynthetic capacity when inhabiting corals in deeper, light-limited environments (Hennige et al. 2008). Furthermore, low-light adapted corals have a high capacity for photosynthetic response to increases in light (Anthony and Hoegh-Guldberg 2003). Thus, the low-light adapted *Cladocopium SU* may have a greater capacity to adjust their photosynthetic activity when exposed to a change in light availability than *Cladocopium WH*.

Contrarily, *Cladocopium WH* was found to transcribe slightly more nitrogen assimilation DEGs than *Cladocopium SU*. This finding is consistent with currently unpublished research on the *Cladocopium WH* transcriptome which found more total DEGs across all transcripts compared to *Cladocopium SU* (Avila-Magaña et al. *in preparation*). Among the nitrogen

assimilation DEGs downregulated in *Cladocopium WH* is a transcript coding for urease. Urease is used to transform host-derived urea into more readily assimilated ammonium and indicates an adaptability for survival in oligotrophic environments (Grover et al. 2006; Ip et al. 2020). A downregulated urease DEG suggests *Cladocopium WH* may be capable of deriving host-derived ammonia only when other photosynthetic and nitrogen assimilation enzymes are functioning properly. Contrarily, previous research has found endosymbiotic Symbiodiniaceae to survive for weeks in dark conditions off host-derived nitrogen (Martinez et al. 2022). Future studies should expand the timeframe in which *Cladocopium SU* and *Cladocopium WH* are exposed to dark conditions to determine whether their long-term survivability under environmental duress is comparable to Symbiodiniaceae in endosymbiotic systems.

Entrainment of Rubisco

Cladocopium SU and *Cladocopium WH* exhibited unexpected significant transcription of *rbcl*, the transcript coding for Rubisco. Unlike transcripts coding for all other photosynthesis enzymes, all five *rbcl* DEGs in *Cladocopium SU* exhibited upregulation between dim and dark conditions and no difference in expression between light and dark conditions. In *Cladocopium WH, rbcl* took an opposite pattern, with an apparent upregulation of expression in dim conditions compared to light and dark conditions. *Cladocopium SU* and *Cladocopium WH* may be exhibiting entrainment, or a fixed pattern, of *rbcl* expression. A strong diel pattern in *rbcl* expression has previously been found in diatoms and cyanobacteria (Paul 1996) and cultured Symbiodiniaceae (Mayfield et al. 2014). Furthermore, photosynthetic rhythm within endosymbiotic Symbiodiniaceae can be entrained by different light clues and is tied to an internal circadian rhythm (Sorek and Levy 2012). Endosymbiotic Symbiodiniaceae have also

shown decreased *rbcl* expression in dim light during the transition from the end of an illumination period to the beginning of a darkness period (Mayfield et al. 2014). While this finding supports the pattern observed in *Cladocopium SU*, it brings into question why *Cladocopium WH* exhibits upregulation when in dim conditions.

Current research proposes a variety of means by which *rbcl* expression may be entrained in *Cladocopium SU* and *Cladocopium WH,* with no definitive answer as to why these patterns happen and why they differ between species. Additionally, it is unknown how long entrainment lasts in dark-exposed *Cladocopium*, important information as the continuation of carbon fixation may be critical to prolonged photosymbiotic success under environmental stress. Cultured Symbiodiniaceae have shown a loss of *rbcl* entrainment after two days of dark exposure (Mayfield et al. 2014). However, the data in this study was collected after three days, suggesting entrainment may last longer in *Cladocopium* inhabiting a host. Future research into the Fraginae system should consistently sample for *rbcl* expression over an extended study period to determine what method of entrainment *Cladocopium SU* and *Cladocopium WH* exhibit*,* if entrainment always follows opposite patterns in these species, and how long entrainment may function as other photosymbiotic processes breakdown.

Conclusion

The Fraginae system represents a subset of photosymbiotic reef species which exhibit exosymbiosis. While endosymbiotic species, such as corals, have been well-researched under a variety of anthropogenic stressors, little is known about the response of exosymbiotic reef species to unprecedented environmental change. This study builds our understanding of exosymbiosis by utilizing transcriptomics to pinpoint photosynthesis and nitrogen assimilation

genes which exhibit differential expression under light stress in *Cladocopium* inhabiting two species of Fraginae cockles, *F. sueziense* and *F. whitleyi*. Photosynthesis and nitrogen assimilation DEGs were found in *Cladocopium* inhabiting both *F. sueziense* and *F. whitleyi*. However, photosynthesis DEGs were more common and represented a greater diversity of proteins in *Cladocopium* inhabiting *F. sueziense.* Contrarily, nitrogen assimilation DEGs occurred slightly more in *Cladocopium* inhabiting *F. whitleyi.* These findings indicate that exosymbiotic *Cladocopium* have photosymbiotic pathways sensitive to environmental stress. Future research may utilize the DEGs found in this study to guide investigations into the response of the *Cladocopium*-Fraginae system to anthropogenic stressors, such as heat stress, ocean acidification, and nutrient enrichment.

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